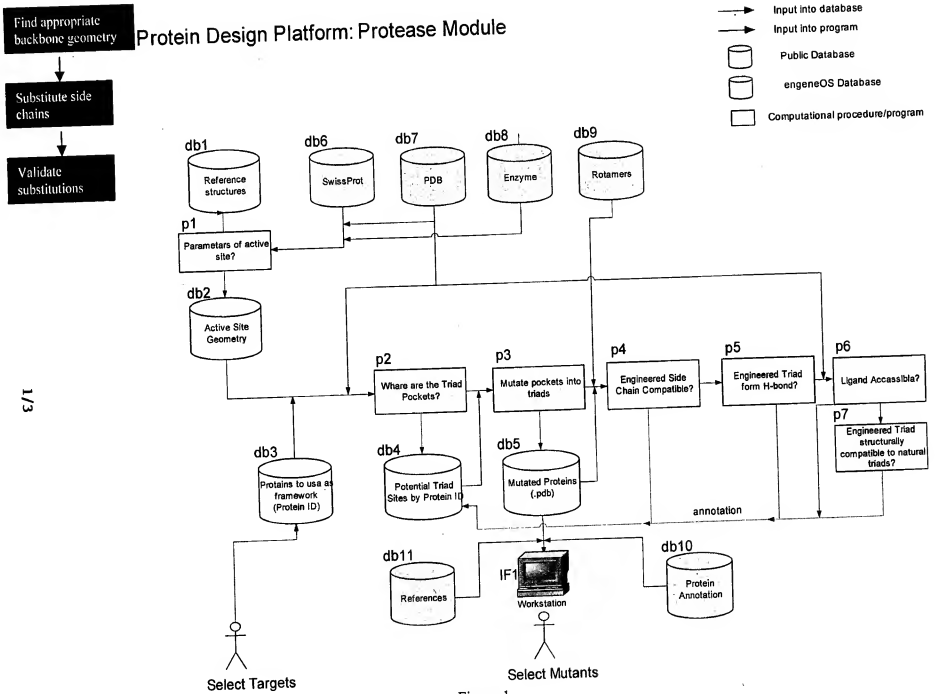
Find appropriate backbone geometry
Substitute side chains
Validate substitutions
Protein Design Platform: Protease Module
Input into database
Input into program
Public Database
engeneOS Database
Computational procedure/program
db1
Reference structures
db6
SwissProt
db7
PDB
db8
Enzyme
db9
Rotamers
p1
Parametars of active site?
db2
Active Site Geometry
p2
Whare are the Triad Pockets?
p3
Mutate pockets into triads
p4
Engineered Side Chain Compatible?
p5
Engineered Triad form H-bond?
p6
Ligand Accassibla?
p7
Engineered Triad structurally compatible to natural triads?
db3
Proteins to usa as framework (Protein ID)
db4
Potential Triad Sites by Protein ID
db5
Mutated Proteins (.pdb)
annotation
db11
References
IF1
Workstation
db10
Protein Annotation
Select Targets
Select Mutants

Figure 1

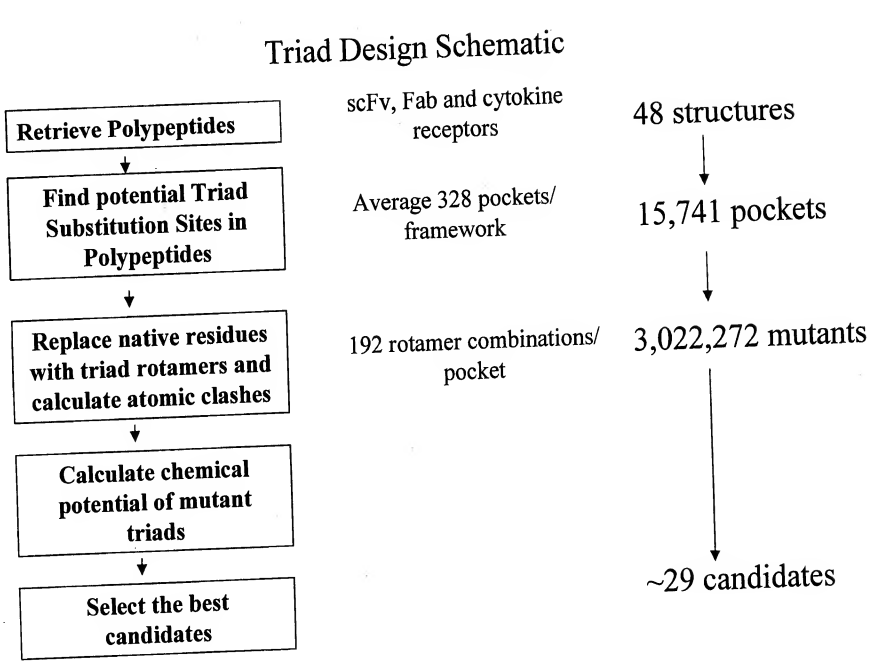
Triad Design Schematic
Retrieve Polypeptides
Find potential Triad Substitution Sites in Polypeptides
Replace native residues with triad rotamers and calculate atomic clashes
Calculate chemical potential of mutant triads
Select the best candidates
scFv, Fab and cytokine receptors
Average 328 pockets/ framework
192 rotamer combinations/ pocket
48 structures
15,741 pockets
3,022,272 mutants
~29 candidates

Figure 2

FIGURE 3

Overall mutation: 1TNF_b_tyr_119_asp, c_tyr_119_his, c_tyr_59_ser

1. Chain B mutation: b_tyr_119_asp

>1TNF_alpha
GTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCCCATGTTGTAGCAAACCCTCAAGCTGAG
GGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGTGGAGCTGAGAGATAAC
CAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACTCCCAGGTCCTCTTCAAGGGCCAAGGCTGC
CCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAAGGTCAAC
CTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTAT
GAGCCCATCGATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGG
CCCGACTATCTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTGA

Resulting Protein sequence, chain B:
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYS
QVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIDL
GGVFQLEKGDRLSAEINRPDYLLFAESGQVYFGIIAL

2. Chain C mutations: c_tyr_119_his, c_tyr_59_ser

>1TNF_alpha
GTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCCCATGTTGTAGCAAACCCTCAAGCTGAG
GGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGTGGAGCTGAGAGATAAC
CAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCAGTTCCCAGGTCCTCTTCAAGGGCCAAGGCTGC
CCCTCCACCCATGTGCTCCTCACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAAGGTCAAC
CTCCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTAT
GAGCCCATCCATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGG
CCCGACTATCTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTGA

Resulting Protein sequence, chain C:
VRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLISS
QVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIHL
GGVFQLEKGDRLSAEINRPDYLLFAESGQVYFGIIAL